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TI ***STABLE*** ***INSULIN*** ***PREPARATION*** FOR IMPLANTED INSULIN PUMPS LABORATORY AND ANIMAL TRIALS.

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Stable Insulin Preparation for Implanted Insulin Pumps

Laboratory and Animal Trials

ULRICH GRAU AND CHRISTOPHER D. SAUDEK

SUMMARY

The stability and longevity of the polyethylene-polypropylene glycol–stabilized insulin have been tested in vitro and in vivo in an implanted insulin-infusion device, the programmable implantable medication system (PIMS). In vitro tests demonstrated long-term compatibility with refill cycles of up to 3 mo, with a preparation of 400 U/ml. Total test period in vitro was 3.2 device-yr (combined time of device use). Insulin retained 88–93% native structure. A major modification, which was biologically active and nonimmunogenic, was identified and partially characterized. Examination of one device by scanning electron microscopy and X-ray microanalysis after 1 yr of insulin infusion revealed surfaces clean of insulin precipitate or other material along the entire insulin-delivery pathway. Surface analysis of the silicone-lined polyethylene catheters after 6 mo of infusion also showed no evidence of major insulin precipitate. In vivo stability trials were accomplished with PIMS implanted in diabetic dogs with an intraperitoneal delivery site. There has been no insulin blockage of the catheter of active pumps after 5.1 dog-yr (combined time of trials) of trials (up to 5 mo between refills in a single dog). Structural stability of insulin was analyzed by high-performance liquid chromatography. On average, 90.8% of the insulin sampled from the reservoir in vivo was native insulin, with an average of 96.2% retention of active forms.

Diabetes **36:1453–59, 1987.**

The stability of insulin has been a significant impediment in the development of mechanical medication-delivery devices for diabetes. An inherently fragile protein, insulin has a tendency to precipitate, aggregate in high-molecular-weight forms, and denature. In external insulin-infusion pumps, catheter blockage is a significant source of clinical complications (1,2), and infusion of altered insulin has been implicated as a cause of elevated serum amyloid protein (3) and even amyloidosis (4), although the latter assertion is controversial (5–7).

The problems associated with insulin use in implantable pumps are even greater, because residence time in the reservoir may be relatively long, thermal exposure is greater, and there is long-term contact with metallic and synthetic surfaces as well as mechanical stresses in the pump itself. To overcome these obstacles, various insulin preparations have been proposed (8–14), including the addition of serum (8), bicarbonate (9), calcium (11), and glycerol (13) to insulin and the use of sulfated insulin (12).

This study describes laboratory and in vivo testing of an insulin preparation specifically formulated for implanted insulin pumps. Hoe 21 PH (10,15), containing polyethylene–polypropylene glycol has been tested in the programmable implantable medication system (PIMS).

MATERIALS AND METHODS

Insulin preparation. Hoe 21 PH is a pH-neutral, buffered insulin formulation containing either 100 or 400 IU/ml semisynthetic human insulin (16), 27.8 or 111 μg/ml zinc ions (for U-100 and U-400 insulin, respectively) with 2 mg/ml phenol as a preservative, 16 mg/ml glycerol as an isotonicity agent, 50 mM of tris-(hydroxymethyl)-aminomethane (Tris) buffer, and 10 μg/ml polyethylene–polypropylene glycol (Genapol, Hoechst AG, Frankfurt, FRG). Genapol is a block polymer of 1800 M_r. The central portion of the molecule consists of propylene glycol units with ~5% (by mass of the polymer) of ethylene glycol units attached at each end. Further details concerning the polymer and its selection for stabilization of insulin are provided in the literature (10,15,17).

Genapol has undergone a series of pharmacologic and toxicologic tests, including acute toxicology and subchronic toxicology on rats and dogs. Doses of Genapol up to 5.0 mg/kg body wt were administered to 120 rats intravenously or subcutaneously. Chronic studies included 3-mo trials in dogs with doses up to 1.0 mg · kg^{-1} · day^{-1}. Embryotoxicity,

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Ames test, micronucleus test, and erythrocyte hemolysis tests were performed. There was no evidence of significant toxicity due to Genapol. These data have been filed with the Food and Drug Administration and comparable German authorities. The final insulin preparation in Hoe 21 PH contains 10 μg/ml Genapol, regardless of the insulin strength, so that a human receiving an average 50 U of U-400 insulin daily would receive 1.25 μg (0.018 μg/kg) Genapol.

Samples from various insulin-manufacturing batches were used. The insulin solution was deaerated before transfer into PIMS by applying a vacuum with a sterile needle for 3 h or more to ensure that the Po_2 was <100 mmHg.

PIMS. The implanted portion of PIMS was a fully programmable, variable rate implanted medication-delivery system. Its titanium casing was a disk, 3 inches in diameter by 0.9 inch thick. When implanted subcutaneously, a refill port was located in the center of the superficial surface. The outlet port delivers insulin to a catheter, the distal tip of which was placed in the peritoneal space. The unit weighed 220 g when the reservoir was full, with catheter attached, and the reservoir volume was ~12 ml.

The PIMS was filled with deaerated insulin through the inlet port with a syringe. The insulin entered an antechamber, passed a filter, and entered the reservoir. The reservoir was separated from a chamber containing Freon by a bellows-type diaphragm. This Freon maintained a −4 psi pressure under physiologic conditions, so that insulin was drawn into the reservoir without external manual pressure, reducing the chance of mistakenly injecting insulin outside the pump. From the reservoir, insulin passed through a one-way valve into the solenoid-pump mechanism, which delivered pulses of 2.0 μl. Throughout its transit in the fluid handling system of PIMS, insulin came in contact primarily with titanium-lined metal surfaces and with the catheter.

The catheter was polyethylene–interior-lined tubing (0.01 inch inside diam) with a Silastic exterior. This was designed after experiments demonstrated that Silastic was the preferred surface to reduce peritoneal reaction, whereas polyethylene was compatible with Hoe 21 PH (17) and was necessary to reduce CO_2 transport from body fluids into the lumen of the catheter. Total catheter length was ~5 inches; a proximal 3-inch portion led to a right-angle bend and a 2-inch distal portion.

Accelerated laboratory testing. Five PIMS devices were mounted on a shaking apparatus (model HT, Braun Melsungen, FRG) and agitated at an amplitude of 2 cm and frequency of 80 cycles/min. Hoe 21 PH was pumped at a constant rate of 11.6 μl/h (28 U/day) with U-100 insulin and 1.68 μl/h (16 U/day) with U-400 insulin. A 20-cm length of the standard catheter material was used, moving freely in air, delivering insulin into a steady glass vial. The vial was changed weekly, and the entire apparatus was maintained at 37°C. Pumps were refilled every 4 wk during the Hoe 21 PH U-100 test period and up to every 12 wk in the Hoe 21 PH U-400 tests.

Insulin sampled from these in vitro tests was compared with Hoe 21 PH insulin maintained without shaking in a glass vial at 37°C for identical periods (see RESULTS).

Analysis of pump and catheter. Laboratory tests included routine analysis of the catheter through which insulin had been infused for 6 mo. The catheter was cut into pieces and inspected via scanning electron microscopy (SEM) and X-ray microanalysis. The samples were dipped in distilled water and then mounted on an electron-microscopy grid, carbon coated, and analyzed in X-ray microanalysis via a module from Princeton Gammatech (Princeton, NJ). Spectra were recorded as an energy-dispersive pattern.

After 12 mo of testing with Hoe 21 PH, one PIMS pump was cut apart to inspect the drug-exposed surfaces of the reservoir and the entire fluid handling system for precipitates. SEM and X-ray microanalysis were performed as described above.

In vivo tests. Eight dogs, each made diabetic by total pancreatectomy 1–3 yr before implantation, were used for in vivo tests. Each pump was implanted subcutaneously in the dog's flank.

Insulin was delivered in a dosage pattern according to clinical need. Basal rates were established for dawn, daytime, and nighttime, with supplemental infusion patterns programmed to cover the 6- to 8-h absorptive period of the dogs. Basal rates varied from 0.2 to 0.8 U/h.

Pumps were refilled transcutaneously when the reservoir was <25% full. In addition, insulin in the reservoir was sampled periodically for analysis (see RESULTS). This was accomplished by use of a syringe with a Whitacre needle (Popper, New Hyde Park, NY) entered into the reservoir, with

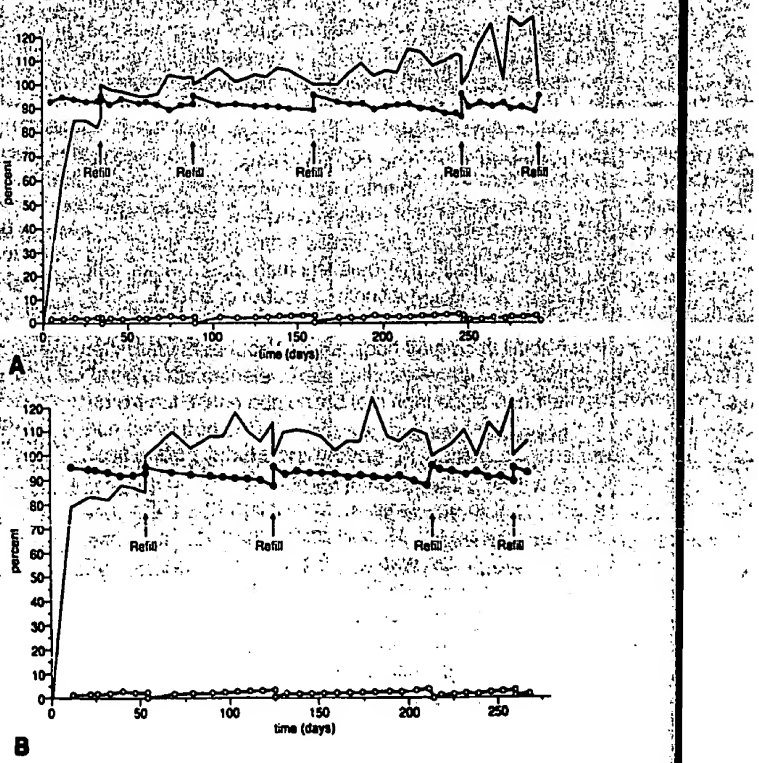

FIG. 1. In vitro tests of Hoe 21 PH U-400 insulin stability in 2 programmable implantable medication systems (*A* and *B*). Samples collected from pump studies were assayed by HPLC. Refills were performed at times indicated by arrows. *Solid line*, total insulin concentration as percent of external insulin standard; ●, percent of total insulin assayed as native insulin by HPLC; ○, percent of total insulin assayed as major modification product (see text).

TABLE 1
Insulin concentration in Hoe 21 PH samples from PIMS in vitro tests based on HPLC analysis in percent of initial concentration

Device	Insulin type	Mean insulin concentration in eluate samples	*n*	Mean insulin quantity in reservoir samples	*n*
1	Hoe 21 PH U-100	98.1 ± 7.6	22	100.4 ± 5.6	5
2	Hoe 21 PH U-100	98.6 ± 7.2	24	96.8 ± 5.5	5
3	Hoe 21 PH U-400	101.3 ± 7.5	12	99.0 ± 5.5	2
4	Hoe 21 PH U-400	96.9 ± 17.5	30	92.3 ± 7.5	3
5	Hoe 21 PH U-400	102.2 ± 12.6	25	89.5 ± 6.4	2
Total		99.4 ± 10.3	113	95.6 ± 5.9	17

Eluate samples were collected continuously in 1-wk aliquots; reservoir was sampled before refills every 4 wk (U-100) or 12 wk (U-400). PIMS, programmable implantable medication system; *n*, number of samplings taken from each device, at the catheter outlet (eluate) or from the device reservoir.

suction applied manually to remove ~0.5 ml of insulin, diluted with ~0.5 ml of insulin-free diluting fluid in the syringe before insertion of the needle into the reservoir (to avoid air entering the reservoir).

Analysis of insulin. Insulin was routinely analyzed by high-performance liquid chromatography (HPLC) as described previously (18). Insulin quantity was determined with reference to an external standard, whereas the structural integrity was assessed by relative distribution and size of individual peaks on the chromatogram. External standards were routinely assessed for equivalent radioimmunoassayable and biologic insulin activity.

Analysis of the major modification product (MMP). Isolation and analysis of the MMP were accomplished by preparative HPLC with a Vydac TP RP 10-µm particle size column of 25 × 0.46 cm (inside diam) dimensions and a mobile phase consisting of an *n*-butanol–ethanol water mixture (19). Fractions were pooled according to the optical-density tracing and lyophilized. The lyophilized powder was assayed for protein via the standard biuret method and by HPLC with reference to an insulin standard. Disk gel electrophoresis and subsequent Coomassie blue staining were performed by standard procedures (20). *Staphylococcus aureus* V8 protease fingerprint analysis was performed as described previously (21).

To test for potential immunogenicity of the MMP, a Hoe 21 PH preparation was recycled repeatedly inside a pump at 37°C, and MMP was generated in large proportions. The insulin was then precipitated, and HPLC analysis revealed 76% of MMP in the final product. Five pigs were hyperimmunized with 0.036 mg/kg of the mixture (corresponding to 1 U/kg) in complete Freund's adjuvant. Boosters were given in weekly intervals, and from the 6th wk on, no adjuvant was added, and the doses were reduced to 0.2 U/kg. A reference group of five pigs was treated accordingly with human insulin. Blood was withdrawn weekly, and the percent binding was determined with a porcine insulin tracer. Values were calculated as means ± SF.

RESULTS

Insulin stability in accelerated laboratory testing. The total in vitro test period with Hoe 21 PH U-100 was 10.7 device-mo (22 and 24 wk in 2 pumps), and with Hoe 21 PH U-400 was 15.6 device-mo (12, 30, and 25 device-wk, in 3 pumps). The length of refill cycles varied between 4 and 12 wk. Insulin collected from the catheter tip of the laboratory-tested devices as well as reservoir contents removed during these tests were uniformly clear on visual inspection.

After an initial rise in the insulin content of the reservoir during replacement of diluting fluid by insulin, there was a slight (3–5%) decrease in percent native insulin over the residence time in the reservoir, corrected by each refill. Figure 1 shows data from the two pumps sampled at regular intervals over 250–300 days. All in vitro data are summarized in Tables 1 and 2. Overall, in vitro tests of Hoe 21 PH U-100 and U-400 combined showed a mean of 99.4% retention of insulin concentration (including modification products) in the eluate and 95.6% retention in the reservoir samples (Table 1; differences between eluate and reservoir were insignificant). Later tests of insulin concentration in U-400 eluate (devices 4 and 5, Table 1) used a flow rate of only 40 µl

TABLE 2
Native insulin content (% ± SD) in Hoe 21 PH samples from PIMS in vitro tests, based on HPLC analysis, as function of residence time in PIMS or in glass vial

Residence time (wk)	Hoe 21 PH U-100				Hoe 21 PH U-400				Hoe 21 PH U-100
	PIMS eluate	*n*	PIMS reservoir	*n*	PIMS eluate	*n*	PIMS reservoir	*n*	Glass vial (steady)
0			97.7 ± 0.8	13			95.7 ± 0.7	9	97.5
1	96.7 ± 0.7	13			93.1 ± 1.9	4			
2	96.4 ± 0.8	12			93.9 ± 1.5	9			96.6
3	96.5 ± 0.8	12			93.4 ± 1.0	9			96.5
4	95.0 ± 1.2	10	95.1 ± 1.2	8	93.4 ± 1.0	7			
5					92.2 ± 1.6	8	93.9 ± 0.1	2	
6					91.8 ± 2.2	8	93.5	1	95.0
7					91.6 ± 0.8	7			
8					91.6 ± 1.1	7	92.9 ± 0.5	3	
9					90.2 ± 0.5	4			
10					89.7 ± 1.7	4	89.4	1	
12					87.7	2			

Samples were taken on weeks indicated; eluates were collected continuously in weekly aliquots. PIMS, programmable implantable medication system.

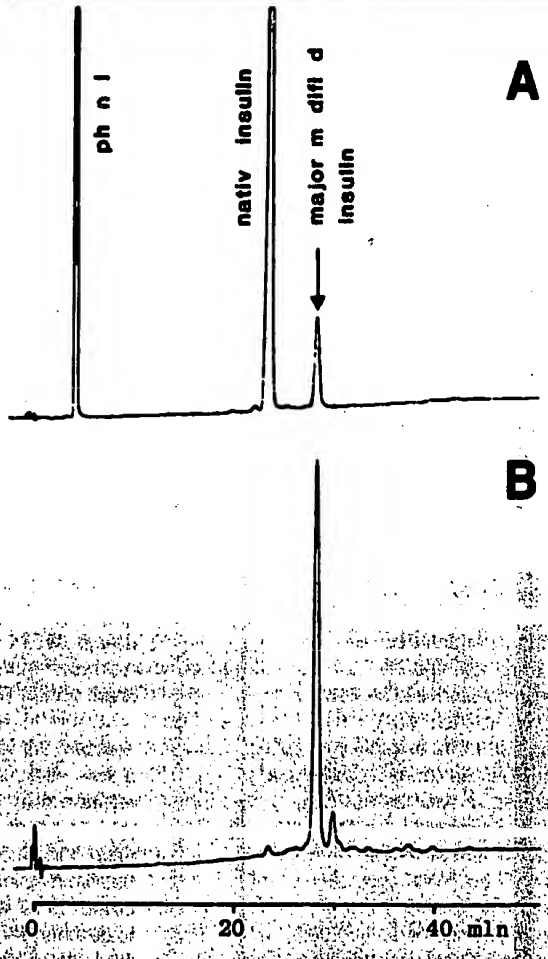

FIG. 2. *A*, typical preparative HPLC tracing of insulin from programmable implantable medication system pump eluate in vitro (see text), showing elution of phenol, native insulin, and major modified product of insulin. *B*, the isolated peak from major modified product of insulin, showing that native insulin was practically absent, whereas desamido-A21 derivative of major modified product, formed during HPLC preparation, is present.

(16 U) per day, increasing the experimental error and variance of the data.

The chemical integrity of insulin sampled both from the reservoir and from the eluate decreased slowly over 12 wk, with exposure to 37°C temperature (Table 2). The changes were comparable to those seen in insulin stored in a glass vial at 37°C without movement (Table 2) and did not differ significantly whether U-100 or U-400 preparations were used. Native insulin accounted for 87.7 to 95.7% of the total insulin concentration at all times (Fig. 1). Up to 3.4% of the total was desamido-A21-insulin (22,23), and the biologically active MMP, described previously to result from residence in pump reservoirs (18), never exceeded 3.3% of the total.

Preparative HPLC of the MMP isolated from PIMS eluate is shown in Fig. 2. The isolated peak of the MMP (Fig. 2*B*) shows that insulin was practically absent, the small peak next to the MMP being its desamido-A21 derivative generated during HPLC purification. This peak assessment is confirmed by the disk gel, which showed the major band comigrating with reference insulin and the minor band comigrating with desamido-A21-insulin. The *S. aureus* proteas V8 fingerprint, which yields three major fragments with human insulin (Fig. 3*A*), gives an identical pattern for the MMP with regard to fragments II and III, but fragment I is eluted significantly later (Fig. 3*B*). Fragment I contains the NH_2-terminal region of the B-chain and residues 5–17 of the A-chain. Identification and assignments of the fragments have been published (21).

The biological potency of the purified MMP was tested on rabbits. The sample had a biological potency of 27.5 U/mg dry wt, not significantly different from the 28 U/mg obtained for the reference human insulin.

Testing for immunogenicity of the pump generated modified insulin, according to the immunization procedure described in MATERIALS AND METHODS, ^{125}I-insulin binding did not differ significantly from a reference group of sera from pigs exposed to regular human insulin (Fig. 4). (Immunization data kindly provided by H.P. Neubauer, Hoechst AG.)

Surface analysis in accelerated laboratory testing. After 12–30 wk of operation with Hoe 21 PH insulin delivery in the accelerated laboratory tests, the inner surfaces of the five catheters as well as the entire fluid handling system of one

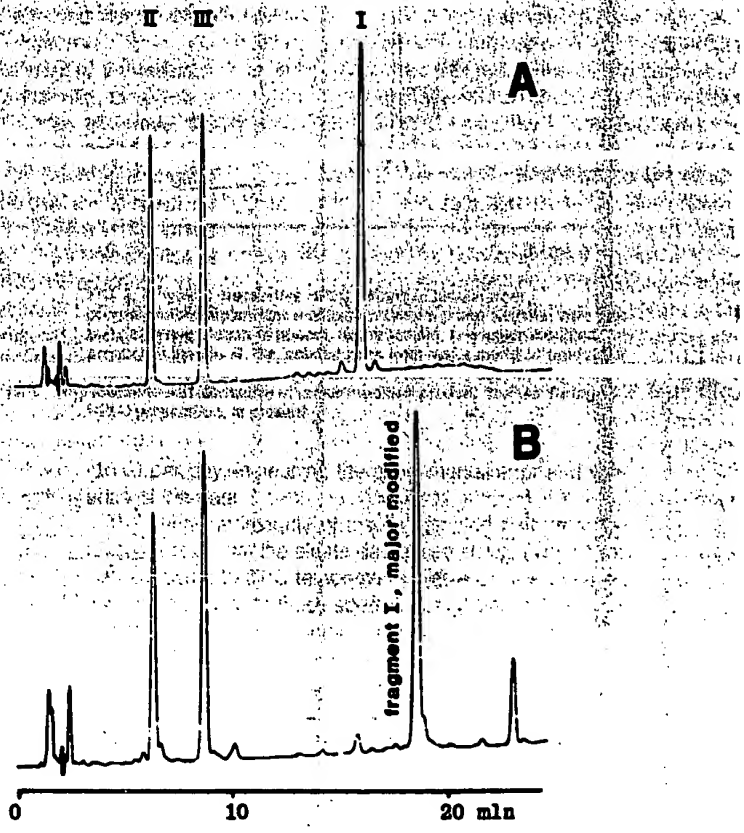

FIG. 3. *A*, *Staphylococcus aureus* protease V8 fingerprint HPLC analysis of human insulin major modification product (MMP) showing 3 major fragments. *B*, *S. aureus* protease V8 fingerprint HPLC analysis of Hoe 21 PH showing identical patterns for MMP from human insulin, with the exception of fragment I, which is eluted significantly later. See ref. 21 for details.

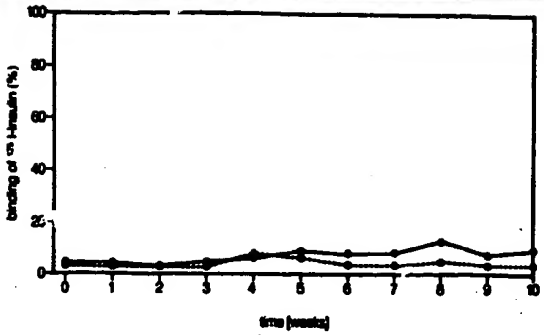

FIG. 4. Binding of ^{125}I-insulin to sera from 5 pigs immunized with insulin taken from pump eluates in vitro (*dashed line*) and insulin unexposed to programmable implantable medication system (*solid line*). See text for details.

PIMS unit were examined at selected representative sites. The sulfur signal in X-ray microanalysis, indicative of insulin due to its six cysteines, was absent in most specimens. Surfaces were clean of apparent precipitate even in remote corners. No major particulate deposits were detected. Traces of insulin were found in few locations, which became visible only if the electron beam was directed at these microscopic patches. In two cases, trace deposition of insulin was detected on the diaphragm and on the valve. A sulfur signal was recorded in the area of the pump outlet-to-catheter connection, where insulin was exposed to ~2 mm silicone. This indicates that there is a surface layer of insulin at the silicone interface, although the insulin is nonparticulate. This silicone junction has been deleted in recent pump designs.

In vivo test results. Table 3 shows the results of in vivo testing with Hoe 21 PH insulins in PIMS-implanted dogs. Since these periodic samplings were completed, there have been an additional 241 dog-wk (combined times) of experience with Hoe 21 PH U-400, with normal functioning of PIMS (i.e., no pump stoppage or catheter block from insulin). Total experience in vivo with U-100 Hoe 21 PH insulin, therefore, is 28.6 dog-mo; total experience with Hoe 21 PH U-400 is 103 dog-mo. The longest interval between refills was 5 mo (dog 4, Table 3); the mean interval was 4.1 wk for U-100 and 14.7 wk for U-400.

In early trials, there was less favorable experience with other formulations of insulin. We noted two instances of insulin precipitation in the lumen of a pure Silastic catheter via Hoe 21 PH insulin. It was determined that these resulted from a change in pH of the intraluminal insulin due to permeation of CO_2. Since modifying the catheter to include a polyethylene interior, we have noted no such problem, presumably because polyethylene is far less permeable to CO_2. One episode of insulin precipitation did occur during the trials described herein after Hoe 21 PH U-400 insulin was inadvertently added to a zinc-containing placebo solution in the reservoir. Chemical analysis of the precipitate revealed that there was zinc in concentration exceeding that allowable to keep Hoe 21 PH in solution. Another episode of catheter block occurred after a catheter was allowed to remain in place for >3 mo without flow. Under normal operating conditions (i.e., without excess zinc added and with normal flow), there has been no episode of insulin precipitation over the combined period of 11-dog-yr in viv trials.

Insulin sampled from the reservoir as described was assayed by HPLC for concentration and structural integrity on 12 occasions, testing samples that had been in the reservoir 31–147 (mean 77) days (Table 3). Comparing these samples with samples drawn simultaneously from vials of insulin that had been refrigerated, Hoe 21 PH insulin retained an average of 92% of its original concentration. Peak analysis of HPLC tracings revealed that on average 90.8% of the material was native insulin, whereas 5.4% was biologically active desamido or major modification product.

Residence time in reservoir did not correlate with loss of total insulin concentration as percent of standard. It is unclear what factors contributed to the irregular reduction in assayed insulin concentration. Possibly, experimental error was introduced by the diluting of sampled insulin in a syringe prefilled with 1–2 ml diluting fluid. There was, however, a negative correlation between time in reservoir and retention of native, unmodified insulin ($r = -.62$, $P < .04$). These in vivo data agree with those of in vitro trials shown in Table 2 and Fig. 1.

Glycemic control of these diabetic dogs was good. Mean plasma glucose of implanted dogs over the entire period of this study was 103 ± 47 mg/dl in the morning and 101 ± 47 mg/dl in the afternoon. There was no trend toward either worse diabetic control or increased insulin dosage between refills, suggesting that the structural changes were not clinically significant. Also, we have never noted an episode of clinical amyloidosis in pump-treated dogs, although one dog who was never exposed to a pump died of renal amyloidosis presumed to be secondary to chronic infection of an indwelling venous catheter.

DISCUSSION

Factors affecting the chemical and physical stability of insulin have been discussed extensively (8–15,18,24–30).

TABLE 3
Insulin concentration and chemical stability from reservoir samples withdrawn sequentially during in vivo dog trials

Dog number	Sample	Days in PIMS at time of sample	Total insulin concentration (% of standard)	Native insulin content (% of total insulin)
3	a	31	100	93.0
4	a	38	85	90.1
	b	91	101	90.3
	c	125	93	93.4
	d	147	98	85.8
5	a	38	95	92.0
	b	85	104	93.8
	c	138	78	88.4
13	a	31	90	93.1
	b	42	100	90.6
28	a	57	77	90.9
	b	104	89	88.3
Mean		77	92	90.8

Insulin concentration, percentage of control Hoe PH 21 unexposed to programmable implantable medication system (PIMS). Chemical stability, native insulin as percentage of total insulin by HPLC. Refill cycles were as described in the text.

Whereas clinical preparations for subcutaneous injection are now uniformly stable and highly purified, insulin for implantable infusion pumps requires further steps to ensure stability. Isoelectric or zinc precipitation of insulin (a regular feature of long-acting preparations for subcutaneous delivery) will block the device, and chemical denaturation will reduce biologic activity.

Lougheed et al. (25) reviewed insulin's inherent tendency to aggregate as large-molecular-weight polymers and factors (i.e., motion, temperature, metal ion contamination, impurities, reduced pH, diluents, and insulin heterogeneity) that have been implicated in causing aggregation. Schade et al. (26) studied electron photomicrographs of precipitates formed when neutral short-acting pork insulin was exposed to an implanted Silastic reservoir. They concluded that at least three forms of precipitate occurred: isoelectrically formed microcrystals, macromolecular precipitates, and linear aggregates of insulin forming fibrillar precipitation. Hutchinson (27) demonstrated gelling as a result of prolonged gentle agitation in external delivery systems, whereas Brennan et al. (28) found aggregation to be a significant problem in altering the flow rate of a closed-loop external insulin-delivery system.

Multiple approaches have been tried to reduce insulin blockage of delivery systems. Albisser et al. (8) used autologous serum; the Infusaid pump (Norwood, MA) has utilized 80% glycerol (13); sulfated insulin has been tried (12); and calcium ion has had some beneficial effect (14). With various surfactant materials, Chawla et al. (29) reduced aggregation in shake tests but found significant extraction of impurities from contacted plastics used in infusion sets. Finally, Lougheed et al. (30) studied the surfactant lysophosphatidylcholine and other surfactants in laboratory testing, finding that long hydrophobic groups are most effective in reducing aggregation and that silicone rubber is the most active material in promoting aggregation.

Long-term exposure of insulin to hydrophobic surfaces such as titanium, although less dangerous than silicone rubber, still poses a difficult problem. The NH_2-terminal amino acids of insulin are thought to adsorb to the hydrophobic metal surface, undergoing irreversible conformational changes, and even desorbing to serve as nucleation centers for larger aggregates (18). Hoe 21 PH was developed to block this sequence.

Genapol, a surface-active polyethylene-polypropylene glycol, effectively prevents adsorption of insulin to hydrophobic surfaces. As analyzed by ESCA spectroscopy (17), a very small portion of the Hoe 21 PH is adsorbed compared with that seen in conventional insulin preparations. This study tests whether these physicochemical analyses are supported by reliable delivery of Hoe 21 PH in an implantable pump (PIMS).

The data demonstrate good stability in accelerated laboratory tests and after as long as 5 mo between refills in vivo. Toxicology testing indicated no adverse sequelae of either intraperitoneal or intravenous routes of delivery, and early clinical trials with Hoe 21 PH have been initiated in humans. However, several caveats are in order. First, each specific material that insulin contacts must be tested. For example, Genapol is far less effective in preventing adsorption to silicone than to polyethylene (17), and we noted slight insulin residue at the 2 mm of silicone touched by insulin at the interface between the outlet port and the catheter hub. We have also shown that the zinc concentration of insulin must be closely maintained to avoid zinc crystallization. Studies reported herein have not addr ssed other hazards of implantable pump use, such as (for PIMS) air locking of the pump if bubbles are allowed to form in the reservoir, or (for any peritoneal delivery device) fibrotic reaction obstructing the catheter tip.

The protein chemical analyses reported herein do not allow an unequivocal identification of the major modification product. In light of the sensitivity of the Asn B3 amide group for modification at neutral pH (24), and consistent with the fingerprint results, it appears likely that the insulin derivative investigated here is also a B3-derivative. However, it cannot be only a desamido product, because if so it should differ from insulin in its gel electrophoretic pattern. A covalent dimeric insulin is not consistent with these data. In regular insulin preparations, however, there is a minor contamination coeluting in HPLC analysis with the pump-generated major modification product. Further investigation will be needed to positively identify the protein, but its clinical significance is probably minor because it has full potency and no additional immunogenicity when compared with native insulin.

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REFERENCES

1. Irsigler K, Kritz H: Long-term continuous intravenous insulin therapy with a portable insulin dosage-regulating apparatus. *Diabetes* 28:196–203, 1979
2. Lougheed WD, Woulfe Flanaga H, Clement JR, Albisser AM: Insulin aggregation in artificial delivery systems. *Diabetologia* 19:1–9, 1980
3. Brownlee M, Cerami A, Li JJ, Vlassara TR, Martin TR, McAdam KPWJ: Association of insulin pump therapy with raised serum amyloid A in type I diabetes mellitus. *Lancet* 1:411–13, 1984
4. Albisser AM, McAdam KPWJ, Perlman K, Carson S, Bahoric A, Williamson JR: Unanticipated amyloidosis in dogs infused with insulin. *Diabetes* 32:1092–101, 1983
5. Koivisto VA, Teppo A-M, Maury CPJ, Taskinen M-R: No evidence of amyloidosis in type I diabetics treated with continuous subcutaneous insulin infusion. *Diabetes* 32:88–90, 1983
6. Mauer SM, Buchwald H, Groppoly FJ, Rohde TD, Wigness BD, Rupp WM, Williamson MW: Failure to find amyloidosis in dogs treated with long-term intravenous insulin delivery by a totally implantable pump. *Diabetologia* 25:448–50, 1983
7. Bending JJ, Pickup JC, Rowe IF, Gallimore R, Tennent G, Keen H, Pepys MB: Continuous subcutaneous insulin infusion does not induce a significant acute phase response of serum amyloid A protein. *Diabetologia* 28:113–15, 1985
8. Albisser AM, Lougheed WD, Perlman K, Bahoric A: Nonaggregating insulin solutions for long-term glucose control in experimental and human diabetes. *Diabetes* 29:241–43, 1980
9. Schade DS, Eaton RP, Spencer W: Implantation of an artificial pancreas—current perspectives. *JAMA* 245:709–10, 1981
10. Grau U, Selpke G, Obermeier R, Thurow H: Stabile Insulinlosungen fur authomatische Dosiergerate. In *Neue Insuline*. Petersen KG, Schluter KJ, Kerp L, Eds. Freiburg, FRG, Freiburger Graphische Betriebe, 1982, p. 411–19

11. Brange J, Havelund S: Insulin pumps and insulin quality—requirements and problems. *Acta Med Scand Suppl* 671:135–38, 1983
12. Nomura M, Zinman B, Bahoric A, Marliss EB, Albisser AM: Intravenous infusions of sulfated insulin normalize plasma glucose levels in pancreatectomized dogs. *Diabetes* 32:788–92, 1983
13. Blackshear PJ, Rohde TD, Palmer JL, Wigness BD, Rupp WM, Buchwald H: Glycerol prevents insulin precipitation and interruption of flow in an implantable insulin infusion pump. *Diabetes Care* 6:387–92, 1983
14. Brange J, Hansen P, Havelund S, Hommel E, Kuhl C: *Abstr. 3rd Workshop EASD Study Group on Artificial Insulin Delivery Systems, Pancreas and Islet Transplantation, Igls, Austria, Feb. 5–7, 1984*
15. Thurow H, Geisen K: Stabilization of dissolved proteins against denaturation at hydrophobic interfaces. *Diabetologia* 27:212–18, 1984
16. Obermeier R, Seipke G: Enzyme-catalyzed semi-syntheses with porcine insulin. *Process Biochem* 19:29–32, 1984
17. Grau U: Insulin stability. In *Continuous Insulin Infusion Therapy: Experience From One Decade*. Hepp KD, Renner R, Eds. Stuttgart, FRG, Schattauer, 1985, p. 33–46
18. Grau U: Chemical stability of insulin in a delivery system environment. *Diabetologia* 28:458–63, 1985
19. Luiken J, Van der Zee R, Welling GW: Structure and activity of proteins after reversed phase high-performance liquid chromatography. *J Chromatogr* 284:482–86, 1984
20. European Pharmacopoeia: *Gel Electrophoresis*. Vol 3. Maisonneuve SA, Ed. St. Ruffine, France, 1975, p. 71–73
21. Grau, U: Fingerprint analysis of insulin and proinsulins. *Diabetes* 34:1174–80, 1985
22. Harfenist EJ, Craig LC: Countercurrent distribution studies of insulin. *J Am Chem Soc* 74:3883–88, 1952
23. Carpenter FH, Crambach A: On the amide content of insulin fractions isolated from partition chromatography and countercurrent distribution. *J Biol Chem* 237:404–10, 1962
24. Brange J, Lankjaer L, Havelund S, Sorensen E: Chemical stability of insulin: neutral insulin solutions (Abstract). *Diabetologia* 25:143, 1983
25. Lougheed WD, Woulfe-Flanagan H, Clement JR, Albisser AM: Insulin aggregation in artificial delivery systems. *Diabetologia* 19:1–9, 1980
26. Schade DS, Eaton RP, DeLongo J, Saland LC, Ladman AJ, Carlson GA: Electron microscopy of insulin precipitates. *Diabetes Care* 5:25–30, 1982
27. Hutchinson KG: Assessment of galling in insulin solutions for infusion pumps. *J Pharm Pharmacol* 37:528–31, 1985
28. Brennan JR, Gebhart SSP, Blackard WG: Pump-induced insulin aggregation: a problem with the Biostator. *Diabetes* 34:353–59, 1985
29. Chawla AS, Hinberg I, Blais P, Johnson D: Aggregation of insulin, containing surfactants, in contact with different materials. *Diabetes* 34:420–24, 1985
30. Lougheed WD, Albisser AM, Martindale HM, Chow JC, Clement JR: Physical stability of insulin formulations. *Diabetes* 32:424–32, 1983